

REMARKS

Claims 31-55 are pending in this application. Claims 31-55 were rejected under 35 U.S.C. § 103.

By this amendment, claims 44-50 and 52-54 have been canceled, claims 31, 36, and 51 have been amended and new claims 56 and 57 have been added without prejudice or disclaimer of any previously claimed subject matter. Support for the amendments can be found, *inter alia*, throughout the specification, for example in Examples at pages 7 and 17. Support for new claims can be found in the specification, for example, at page 2, lines 25-34. The amendments and new claims involve subject matter already under examination. Accordingly, Applicants respectfully request entry of this amendment.

The amendments are made solely to promote prosecution without prejudice or disclaimer of any previously claimed subject matter. With respect to all amendments and cancelled claims, Applicants have not dedicated or abandoned any unclaimed subject matter and moreover have not acquiesced to any rejections and/or objections made by the Patent Office. Applicants expressly reserve the right to pursue prosecution of any presently excluded subject matter or claim embodiments in one or more future continuation and/or divisional application(s).

Applicants have carefully considered the points raised in the Office Action and believe that the Examiner's concerns have been addressed as described herein, thereby placing this case into condition for allowance.

Finality of the Office Action

The Examiner states that "Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, this action is made final. See MPEP §706.07(a)." Office Action, page 4. Applicants respectfully disagree with the determination of finality for the Office Action.

The currently pending independent claims were all dependent claims rewritten as independent in the response and amendment filed March 4, 2004. These dependent claims had not been subject to the current rejection. Thus, Applicants respectfully submit that the outstanding rejection in the Final Office Action is a new ground of rejection that was neither necessitated by Applicants' amendment of the claims nor based on information submitted in an information disclosure statement filed during the period set forth in 37 C.F.R. §1.97(c). As such, the pending claims should not be subject to a final Office Action. M.P.E.P. §706.07(a).

Accordingly, Applicants respectfully request withdrawal of the finality of the Office Action.

Rejection under 35 U.S.C. §103

Claims 31-55 were rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Lavanchy, *et al.* (1996, *J. Clinical Laboratory Analysis* 10(5):269-276, "Lavanchy"), Lee, *et al.* (1995, *Transfusion* 35:845-849, "Lee") Rosa, *et al.*, (1995, *J. Virol. Methods* 55:219-232, "Rosa"), and Wang *et al.* (U.S. Pat. No. 5,106,726, "Wang"). Applicants respectfully traverse this rejection.

As an initial matter, the Lavanchy reference was published in the fifth of the six issues of *J. Clinical Laboratory Analysis* in 1996 and the publication date of that issue was September 2, 1996. Applicants respectfully point out that the present invention claims priority to May 7, 1996, the filing date of Japanese Patent Application No. 8-112442. Thus, Lavanchy is not a citable reference under 35 U.S.C. §102 and so is not available for this rejection. Accordingly, Applicants will not respond further to this citation.

A *prima facie* case of obviousness requires that three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable

expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20USPQ2d 1438 (Fed. Cir. 1991); MPEP §2143. If any one of these three criteria is not met, a *prima facie* case of obviousness has not been established. As presented below, Applicants respectfully submit that a *prima facie* case of obviousness has not been established.

As amended herein, the claimed invention is directed to a diagnostic reagent for hepatitis C virus (HCV) infection comprising a solid phase sensitized with a mixture of a genetic recombinant HCV antigen and synthesized HCV antigens comprising core, NS4 and NS5 peptides. The claimed invention is also directed to a diagnostic reagent for HCV infection comprising a solid phase sensitized with a mixture of a genetic recombinant HCV antigen and one or more synthesized HCV antigens conjugated with a carrier protein.

Lee describes use of a third-generation HCV screening assay produced by Chiron Corporation referred to as HCV RIBA 3.0. As described on page 846, left column, this is an immunoblot strip assay which contains synthetic NS4 and core peptides and recombinant NS3 and NS5 proteins. In the strip immunoassay of Lee, each of the HCV antigens are immobilized on the test strip in individual bands, i.e., the antigens are not used as an antigen mixture.¹ The assay results of individual antigens are shown in Tables 3 and 4 of Lee. Thus, Lee does not describe or suggest an assay of a mixture of HCV antigens. In addition, Lee does not mention or suggest the use of a synthetic NS5 peptide, either alone or in combination with other antigens. Lee also does not teach or suggest the conjugation of a synthetic HCV antigen to a carrier protein.

Rosa describes a prototype ELISA for serodiagnosis of HCV which involves a combination of a synthetic NS4-NS5 chimeric antigen and a recombinant core-NS3 chimeric antigen. As noted by the Examiner, at page 230, Rosa suggests the use of synthetic NS5 peptides in an attempt to avoid false reactivity associated with some NS5 antigen preparations. Notably, Rosa makes no mention of any false reactivity associated with core or NS3 antigens. Rosa does not

¹ See, Exhibit A for the product explanation for Chiron Corporation's HCV RIBA 3.0 assay described in Lee. This information was obtained from the U.S. Food and Drug Administration web-site: <http://www.fda.gov/cber/label/hcvchir021199LB.pdf>. Pages 1 and 2 provide an explanation of the assay and page 12 provides an illustration of the test strip with the individual antigens indicated.

describe or suggest the use of a synthetic core peptide nor the conjugation of a carrier protein to the chimeric antigens. Thus, Rosa does not teach or suggest the mixture of HCV antigens as claimed.

Wang describes the use of synthetic NS-3 peptides and synthetic core peptides in an immunoassay-based HCV detection method. The Examiner points to columns 34 and 35 of Wang as describing conjugation of the synthetic peptides to BSA and the absorption of the conjugated peptides onto a solid phase, such as erythrocytes and particles. Wang, however, does not teach or suggest the use of a mixture of a recombinant HCV antigen and synthetic HCV antigen as claimed.

The Examiner asserts that it would have been obvious to one of ordinary skill in the art to be motivated by the cited references to use the claimed detecting agent. Office Action, pages 3-4. Applicants disagree with this assertion.

Although various HCV antigen proteins are known in the art, Applicants respectfully submit that there is no motivation in the references or in the art to make a diagnostic reagent with the particular combination HCV antigens as claimed. The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990). The Examiner has not provided support with reasonable specificity for suggestion or motivation for a skilled artisan to modify the teachings of the cited references to substitute the antigens taught in the references with the particular antigen mixture in the claimed invention. There is no suggestion in Lee, Rosa or Wang that the diagnostic reagents described therein are in any way unsatisfactory, accordingly, there is no reason for the skilled person to have substituted the claimed antigens for those of the references. This rejection appears to be based on hindsight in view of the present specification. It is only the existence of benefits associated with the claimed reagent as described in the present specification that the skilled person would have become motivated to explore the use of alternatives to that in the references.

Applicants respectfully submit that there is no motivation or suggestion to combine Lee with Rosa and Wang. Since Lee teaches an assay using individual antigens, i.e., not an antigen

mixture, one of skill in the art would not have been motivated to look to Lee for guidance regarding a detection assay that uses an antigen mixture. In addition, Lee's results with individual antigens does not provide a skilled artisan any expectation of success for a reagent made of a mixture of antigens as claimed.

Thus, Applicants respectfully submit that a *prima facie* case of obviousness has not been established.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. §103.

CONCLUSION

Applicants believe that all issues raised in the Office Action have been properly addressed in this response. Accordingly, reconsideration and allowance of the pending claims is respectfully requested. If the Examiner feels that a telephone interview would serve to facilitate resolution of any outstanding issues, the Examiner is encouraged to contact Applicants' representative at the telephone number below.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, Applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 322732000401.

Dated: October 15, 2004

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EXHIBIT A

Attached: Hepatitis C Virus Encoded Antigen (Recombinant c33c and NS5 antigens; Synthetic 5-1-1, c100, and c22 peptides), CHIRON* RIBA* HCV 3.0 SIA, December 1998, 25 pages.

This information was obtained from the U.S. Food and Drug Administration web-site:
<http://www.fda.gov/cber/label/hcvchir021199LB.pdf>.

**Hepatitis C Virus Encoded Antigen
(Recombinant c33c and NS5 antigens;
Synthetic 5-1-1, c100, and c22 peptides)**

CHIRON* RIBA* HCV 3.0 SIA

**Strip Immunoblot Assay (SIA) for the Detection of
Antibodies to Hepatitis C Virus (Anti-HCV)
in Human Serum or Plasma**

For In Vitro Diagnostic Use

* Trademark

Date Issued: December, 1998

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Hepatitis C Virus Encoded Antigen (Recombinant c33c and NS5 antigens; Synthetic 5-1-1, c100, and c22 peptides)

CHIRON® RIBA® HCV 3.0 SIA

Strip Immunoblot Assay (SIA) for the Detection of Antibodies to Hepatitis C Virus (Anti-HCV) in Human Serum or Plasma

1. Name and Intended Use

The CHIRON® RIBA® HCV 3.0 SIA is an *in vitro* qualitative enzyme immunoblot assay for the detection of antibodies to individual proteins encoded by the hepatitis C virus (anti-HCV) in human serum or plasma. It is intended for use as an additional, more specific test on human serum or plasma specimens found to be repeatedly reactive using a licensed anti-HCV screening procedure, such as Enzyme-Linked Immunosorbant Assay (ELISA). Additionally, the CHIRON® RIBA® HCV 3.0 SIA may be used as an aid in the differential diagnosis of patients with biochemical evidence of hepatitis.

2. Summary and Explanation of the Test

The strip immunoblot assay (SIA), as described by Ebeling¹ and others,² has shown usefulness in elucidating the specificity of the antibody response to the hepatitis C virus (HCV). Detection of anti-HCV by SIA methodology is based upon traditional Western and dot blotting techniques, in which specific immunogens (i.e., antigenic polypeptides) encoded by the HCV genome are immobilized onto a membrane support.^{3,4} Visualization of anti-HCV reactivity in specimens to the individual HCV-encoded proteins is accomplished using an anti-human IgG enzyme-conjugate in conjunction with a colorimetric enzyme substrate.

The CHIRON® RIBA® HCV 3.0 SIA is an *in vitro* qualitative immunoblot assay which utilizes recombinant HCV-encoded antigens and synthetic HCV-encoded peptides immobilized as individual bands onto test strips. The two recombinant antigens (c33c and NS5) and two of the synthetic peptides (c100p and 5-1-1p) are derived from putative nonstructural regions of the virus, while the third peptide (c22p) corresponds to the putative nucleocapsid (core) viral protein. (A graphic representation of the putative HCV genome including the locations of the HCV-encoded antigens and peptides is shown in Figure 1, page 2.) Since the recombinant HCV c33c and NS5 antigens are produced as individual fusion proteins with human superoxide dismutase (hSOD), recombinant hSOD has also been included as a control band on the strip. The hSOD control band enables detection of antibodies against hSOD which are not specific for the HCV-encoded portions of the recombinant HCV antigens. The HCV c33c antigen is produced in genetically engineered bacteria (*E. coli*), while the HCV NS5 antigen and hSOD are produced in genetically engineered yeast (*S. cerevisiae*).

Numerous articles have been published demonstrating the value of earlier recombinant antigen-based RIBA® HCV SIAs as supplemental assays to single- and multi-antigen anti-HCV screening procedures.^{1, 5-13} However, a proportion of specimens repeatedly reactive by a multi-antigen anti-HCV screening procedure are rated as indeterminate by CHIRON® RIBA® HCV 2.0

SIA. The CHIRON® RIBA® HCV 3.0 SIA can provide additional information on such indeterminate specimens.

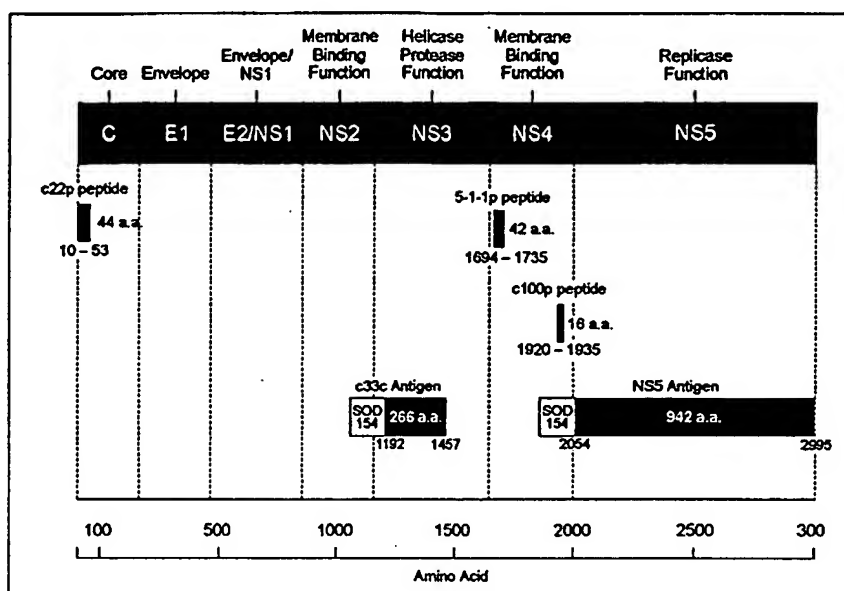


Figure 1

It has been reported that Non-A, Non-B (NANB) hepatitis accounts for 80-90% of post-transfusion hepatitis.^{14, 15, 16} The most significant feature of this form of hepatitis is that about half of the patients infected develop chronic hepatitis, of which 20% may develop cirrhosis.¹⁷ The existence of a transmissible NANB agent has been demonstrated by inoculation of chimpanzees with serum from a chronic NANB hepatitis patient followed by serial passage of the virus into other chimpanzees.^{18, 19}

A strict definition of NANB hepatitis includes the serologic exclusion of hepatitis A, hepatitis B, hepatitis delta, cytomegalovirus and Epstein-Barr virus infection, as well as the exclusion of other causes of liver inflammation.^{20, 21} NANB infection is generally mild, being anicteric in 75% of the cases and symptomatically mild in an even higher percentage, but NANB hepatitis can also be fulminant.²² Mathiesen *et al* have shown that in 36% of all fulminant hepatitis cases NANB infection was diagnosed, and the rate of survival was lower than for fulminant hepatitis A and hepatitis B cases.²³

The genome of a NANB hepatitis agent has been cloned from the plasma of an experimentally infected chimpanzee with a proven high titer of the agent.²⁴ The agent has been designated as hepatitis C virus (HCV). Recombinant HCV-encoded proteins have been utilized to develop first generation (i.e., single-antigen) and second generation (i.e., multi-antigen) screening assays for the detection of anti-HCV in human serum or plasma.^{25, 26} Data from well-characterized NANB hepatitis panels indicate a specific association of HCV antibody with both post-transfusion and community-acquired NANB hepatitis.^{27, 28}

The CHIRON® RIBA® HCV 3.0 SIA, when used as directed in this insert, can detect antibodies to HCV in human serum or plasma. The individual HCV antigen bands present on the test strips allow identification of anti-HCV reactivity to be associated with specific putative viral antigens.

3. Chemical and Biological Principles of the Procedure

The CHIRON® RIBA® HCV 3.0 SIA is a three-stage test which utilizes individual recombinant HCV antigens and synthetic peptides immobilized as individual bands onto the test strips.

In the first stage, the specimen or assay control is diluted and incubated with the strip. Antibodies specific to HCV, if present, will bind to the corresponding recombinant antigen and/or synthetic peptide bands on the strip. Removal of unbound serum/plasma components is accomplished by aspiration and washing.

In the second stage, the strip is incubated in the presence of a peroxidase-labeled goat anti-human IgG conjugate. The conjugate binds to the human IgG portion of the antigen-antibody complexes. Removal of unbound conjugate is accomplished by decantation and subsequent wash steps.

In the third stage, a colorimetric enzyme detection system composed of hydrogen peroxide and 4-chloro-1-naphthol is added. If bound conjugate is present, the enzymatic reaction will produce an insoluble blue-black colored reaction product at each specific HCV antigen, peptide or control band. The color reaction involves the initial divalent oxidation of the peroxidase enzyme by hydrogen peroxide. Subsequent reduction of peroxidase to its initial state by two successive univalent interactions with soluble 4-chloro-1-naphthol results in the insoluble blue-black colored reaction product. After the development of color on the strip, the reaction is stopped by removal of the reactants and final wash steps. The visual band patterns which develop on each individual strip are the result of specific antibody being bound to each of the individual recombinant antigens and/or synthetic peptides on that strip. The reactivity of specimens towards each antigen band is determined by visually comparing the intensity of the individual antigen band with that of the low and high human IgG internal control bands blotted onto each strip (see strip schematic, Figure 3, page 12).

4. Reagents

The CHIRON® RIBA® HCV 3.0 Strip Immunoblot Assay is supplied as a 30-test kit (Product Code 930600).

Component Description	Quantity Provided
STRIP Hepatitis C Virus (HCV) Encoded Antigen (Recombinant c33c and NS5 antigens; Synthetic 5-1-1, c100, and c22 peptides) Coated Strips: each strip contains four individual bands coated with HCV-encoded antigens/peptides, a recombinant human SOD band, and two IgG control bands.	30 consecutively numbered strips: Provided in 6 sealed pouches; each pouch contains 5 strips in separate tubes.
SD Specimen Diluent: phosphate-buffered saline (PBS) with bovine protein stabilizers and detergents. Contains 0.1% sodium azide and 0.05% gentamicin sulfate as preservatives.	1 bottle containing 100 mL
CON Conjugate: peroxidase-labeled goat antihuman IgG (heavy and light chains), with bovine protein stabilizers. Contains 0.01% thimerosal as a preservative.	1 bottle containing 65 mL
4CN Substrate Solution: 4-chloro-1-naphthol in methanol.	1 bottle containing 12 mL
SB Substrate Buffer: phosphate-buffered hydrogen peroxide.	1 bottle containing 60 mL
WB Wash Buffer Concentrate (50x): phosphate-buffered detergent solution containing 0.01% thimerosal as a preservative.	1 bottle containing 80 mL
PC Positive Control (Human): inactivated human serum or plasma containing antibodies to HCV (anti-HCV); nonreactive for hepatitis B surface antigen (HBsAg), antibodies to human immunodeficiency virus type 1 (anti-HIV-1) and type 2 (anti-HIV-2), and antibodies to human T lymphotropic virus type I (anti-HTLV-I) and type II (anti-HTLV-II) when tested by FDA-licensed assays. Contains 0.1% sodium azide and 0.05% gentamicin sulfate as preservatives.	One vial containing 0.3 mL
NC Negative Control (Human): human serum or plasma nonreactive for HBsAg, anti-HIV-1, anti-HIV-2, anti-HCV, anti-HTLV-I, and anti-HTLV-II when tested by FDA-licensed assays. Contains 0.1% sodium azide and 0.05% gentamicin sulfate as preservatives.	One vial containing 0.3 mL

CAUTION: HANDLE AS IF CAPABLE OF TRANSMITTING AN INFECTIOUS AGENT.

Store at 2° to 8°C (Do not freeze).

For *in vitro* Diagnostic Use.

The CHIRON® RIBA® HCV 3.0 SIA meets the FDA potency requirements.

5. Precautions

1. **HANDLE TEST SPECIMENS AND KIT CONTROLS AS IF CAPABLE OF TRANSMITTING AN INFECTIOUS AGENT.** Serum/plasma used to produce the Positive Control was treated to inactivate the hepatitis C virus. In addition, the serum/plasma used to produce the Positive and Negative Controls was shown to be nonreactive for hepatitis B surface antigen (HBsAg), antibodies to human immunodeficiency virus type 1 (anti-HIV-1) and type 2 (anti-HIV-2), and antibodies to human T lymphotropic virus type I (anti-HTLV-I) and type II (anti-HTLV-II) when tested by FDA-licensed assays. However, no known inactivation process or test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, these components must be handled as if they are capable of transmitting infectious agents. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.^{29, 30, 31}
2. Do not pipet by mouth.
3. Do not eat, drink, smoke, or apply cosmetics in areas where specimens or kit components are handled.
4. Wear disposable gloves throughout the test procedure and thoroughly wash hands afterward. Dispose of gloves as biohazardous waste.
5. Disinfect spills promptly with a 0.5% sodium hypochlorite solution (a freshly prepared 1:10 dilution of liquid household bleach) or equivalent disinfectant. Contaminated materials should be disposed of as biohazardous waste.^{29, 30, 32, 33}
6. Any potentially infectious materials, including the Positive and Negative Controls, human specimens, and materials that may come in contact with any of the above, should be handled and disposed as biohazardous waste in accordance with applicable local, state, and federal requirements.^{21, 29, 31, 32, 33} Subject to applicable local, state and federal waste disposal requirements,³⁴ liquid biohazardous waste, including the aspirate from the assay, may be disinfected in a 0.5% sodium hypochlorite solution (a solution with a final volume of a 1:10 dilution of household bleach) prior to sanitary sewer discharge.
7. Components of the kit containing methanol or thimerosal, and waste streams containing these substances, may potentially be classified as hazardous waste.³⁴ Therefore, they should be disposed of in accordance with applicable local, state, and federal waste disposal requirements.
8. The Positive and Negative Controls and the Specimen Diluent contain sodium azide as a preservative. Sodium azide has been reported to react with lead or copper plumbing to form potentially explosive metal azides. Therefore, do not use metal tubing for laboratory apparatus, and always flush an adequate volume of water to prevent metal azide buildup in plumbing systems.³⁵
9. Avoid contact of Substrate Solution (4-chloro-1-naphthol) with eyes, skin, or clothing. If Substrate Solution should come into contact with eyes or skin, wash thoroughly with water. **CAUTION:** The 4-chloro-1-naphthol in the Substrate Solution is a hazardous material and should be disposed of in accordance with all local, state, and federal regulations. **CAUTION:** The Substrate Solution contains methanol. Highly flammable. Toxic by inhalation and if swallowed. Keep container tightly closed. Keep away from sources of ignition. No smoking. Avoid contact with skin. In case of accident, or if you feel unwell, seek medical advice immediately (show label where possible).
10. Bring all kit reagents and samples to room temperature (15° to 30°C) before use (approximately 30 minutes), and return unused reagents to 2° to 8°C after use.
11. **DO NOT INTERCHANGE REAGENTS (i.e., KIT COMPONENTS) AND/OR STRIPS BETWEEN KIT LOTS.** Kit Components and Strips from kits with different lot numbers must not be used together.

12. Do not substitute the Positive and Negative Controls supplied with the kit.
13. **DO NOT CUT STRIPS.** Narrower strips could lead to misinterpretation. Any artifacts which may appear in the reaction zones could be mistaken for reactive bands or may prevent recognition of reactive bands.
14. Do not use kit beyond its expiration date. The date is printed on kit boxes.
15. Test strips and assay tubes may be used only once. Do not reuse test strips or assay tubes once they have been assayed by this test.
16. Do not interchange vial or bottle caps and stoppers; this will lead to cross-contamination of reagents.
17. Do not allow strips to dry during the strip processing steps (steps 5 through 18 of Assay Procedure).
18. To prevent fading, keep developed strips out of strong light (e.g., direct sunlight) and away from heat (greater than 30°C).
19. Do not allow sodium hypochlorite vapors from waste vessels containing chlorine bleach or other sources to contact the strips during the assay as the color reaction may be inhibited.
20. Handle strips gently. Wear gloves throughout the procedure and use forceps to prevent contamination of strips.
21. Distilled or deionized water must be used for preparation of the Working Wash Buffer. Clinical laboratory reagent water, Type I or Type II, is acceptable.* Store water and Working Wash Buffer in nonmetallic containers.
22. Verify that adequate amounts of the Working Wash Buffer and Working Substrate have been prepared before using these reagents in the Assay Procedure. If during the course of the Assay Procedure it is determined that not enough Working Wash Buffer or Working Substrate has been prepared to complete the Assay Procedure, the assay run is invalid and must be repeated using *one* preparation of the Working Solution(s).
23. All pipetting devices should be used with care and calibrated regularly following the manufacturer's instructions.
24. Use individual pipet tips for each specimen to prevent cross-contamination.
25. The maximum run size is 30 tests. Each run must proceed to completion without interruption as specified in the Assay Procedure.
26. Include a kit Positive and Negative Control with each assay run. Treat the Positive and Negative Controls exactly as patient/donor specimens throughout the Assay Procedure (as detailed in Section 10).
27. Failure to add an aliquot of the test specimen as instructed in the Assay Procedure will result in a strip that will be interpreted as negative, regardless if the original specimen is positive or negative for anti-HCV.
28. Failure to adhere to the instructions in this package insert may result in an erroneous test result.

6. Preparation of Reagents

BEFORE USE, BRING ALL REAGENTS TO ROOM TEMPERATURE (15° to 30°C) AND MIX THOROUGHLY BY GENTLY INVERTING CONTAINER SEVERAL TIMES. AVOID FOAMING.

Procedure

1. **Preparation of Working Wash Buffer:** In a clean glass or polypropylene container, prepare sufficient Working Wash Buffer by following the instructions in Table 1. Mix well. The

Working Wash Buffer is stable for 1 week at room temperature (15° to 30°C). Record the preparation date and expiration date of the Working Wash Buffer on the container.

Note: *The Wash Buffer Concentrate contains detergent. During the washing step, residual foam may remain in the wash vessel. The presence of residual foam, however, will not affect the performance of the assay.*

Table 1. Working Wash Buffer Preparation
Reagents for various numbers of strips

Number of Strips	Volume of Wash Buffer Concentrate	Volume of DI ¹ Water
3-5	12 mL	588 mL
6-10	13 mL	637 mL
11-20	15 mL	735 mL
21-30	17 mL	833 mL

¹ Distilled or deionized water.

2. **Preparation of Working Substrate:** In a clean glass or polypropylene container, prepare sufficient Working Substrate for the assay (a minimum of 1 mL per sample, a minimum of 10 mL per wash vessel, and a maximum of 20 strips per wash vessel) by following the instructions in Table 2. The Working Substrate can be prepared up to 1 hour prior to use; store the prepared Working Substrate in the dark. Any unused Working Substrate cannot be stored for subsequent assays. 4-chloro-1-naphthol in the Substrate Solution is a hazardous material and must be disposed of in accordance with local, state, and federal regulations. Record the preparation and expiration date and time of the Working Substrate on the container.

Table 2. Working Substrate Preparation
Reagents required for various numbers of strips

Number of Strips	Volume of Substrate Solution	Volume of Substrate Buffer
3-10	1.7 mL	8.5 mL
11	1.9 mL	9.5 mL
12	2.1 mL	10.5 mL
13	2.2 mL	11.0 mL
14	2.4 mL	12.0 mL
15	2.6 mL	13.0 mL
16	2.7 mL	13.5 mL
17	2.9 mL	14.5 mL
18	3.1 mL	15.5 mL
19	3.2 mL	16.0 mL
20 ¹	3.4 mL	17.0 mL

¹ Maximum number of strips per wash vessel.

7. Storage Instructions

1. Store the kits and/or individual reagents (i.e., kit components) at 2° to 8°C. Do not freeze.
2. Do not use kits and/or individual reagents (i.e., kit components) beyond the expiration date, which is printed on kit boxes and individual component labels.
3. Strips should be used within two weeks after opening the pouch. Unused strips should be left in their original tubes, at 2° to 8°C, in a folded and resealed foil pouch with desiccant. Use tape to reseal pouches, and record the date the pouches were opened and the expiration date of the strips on the pouch.
4. The Working Wash Buffer is stable for one week at room temperature (15° to 30°C).
5. The Working Substrate is stable for one hour at room temperature (15° to 30°C) when stored in the dark.

8. Indications of Instability or Deterioration of Reagents

1. The coated strips are sealed in protective pouches with a humidity indicating desiccant. The desiccant, which is normally blue/purple in color, will become pink if excess moisture is present in the pouch. If the desiccant is pink, the strips should not be used.
2. The Substrate Solution should be colorless. If it appears yellow in color, it has become oxidized and should not be used.
3. Changes in the physical appearance of the reagents supplied may indicate instability or deterioration of these materials. If changes in the physical appearance of the reagents are observed (e.g., obvious changes in reagent color or cloudiness apparent with microbial contamination), they should not be used.

9. Specimen Collection and Preparation

No special preparation of the patient/donor is required prior to specimen collection. However, the following guidelines should be used for collecting specimens for testing by the CHIRON® RIBA® HCV 3.0 SIA:

1. Blood should be collected by approved medical techniques.
2. Specimens that have been heat-treated for up to one hour at up to 56°C may be used.
3. Serum or plasma may be stored at 2° to 8°C for up to one week. Archived specimens (i.e., specimens stored for longer than one week) should be stored frozen (–20°C or colder) to limit possible contamination. **Do not store specimens in self-defrosting freezers.** Frozen specimens should first be allowed to thaw completely (room temperature), then mixed well, and centrifuged if necessary to remove any visible particulate matter. Before testing, allow specimens to come to room temperature. Avoid repeated freezing and thawing of specimens.
4. Clear, nonhemolyzed specimens are preferred. Precipitates in specimens should be removed by centrifugation.
5. No effect on specimen reactivity has been observed when anti-HCV positive and negative specimens with the following potentially interfering substances were tested: hemoglobin (up to 80 mg/dL), triglycerides (up to 1600 mg/dL), bilirubin (up to 60 mg/dL). No effect on reactivity was seen in anti-HCV positive and negative specimens when subjected to up to five freeze-thaw cycles or when spiked with *Candida albicans*, *Staphylococcus epidermidis*, or *Pseudomonas aeruginosa* (each at a final concentration of 10³ CFU/mL) and stored at 2° to 8°C for eight days.

6. Serum (collected in a serum separator tube or evacuated specimen collection tube) or plasma collected by approved techniques in K₂ EDTA (15% solution), sodium heparin, ACD (Solution B), CPDA-I, or 4% sodium citrate may be used. To ensure the correct ratio of anticoagulant to specimen, specimens drawn into evacuated tubes should be evaluated for fill adequacy based on either NCCLS standards or the blood tube manufacturer's instructions.³⁷
7. Specimens, such as cadaveric fluids (including serum and plasma), pleural fluid, saliva, urine, and nonhuman specimens, have not been evaluated in this assay and should not be used.
8. All blood specimens should be handled as if capable of transmitting infectious agents. It is recommended that all handling of blood and blood components be in compliance with the OSHA Bloodborne Pathogen Standard.³¹

If specimens are shipped, they must be packaged in compliance with federal regulations covering the transport of "diagnostic specimens" or "infectious substance"/"etiologic agents" as appropriate to the material and mode of transport.³⁸⁻⁴⁰ Specimens may be shipped refrigerated at 2°-8°C for up to seven days or can be shipped on dry ice. After arrival in the laboratory, specimens shipped at 2°-8°C should be refrigerated at 2°-8°C if they will be tested within seven days of collection. Specimens shipped on dry ice should be placed in a non-defrosting freezer at -20°C or colder until they are ready to be tested.

10. Procedure

A. Materials Provided (30-test kit; Product Code 930600)

Each CHIRON® RIBA® HCV 3.0 SIA kit contains:		
• HCV Encoded Antigen/Peptide Coated Strips (STRIP)	30 strips	6 pouches
• Specimen Diluent (SD)	1 bottle	100 mL
• Conjugate (CON)	1 bottle	65 mL
• Substrate Solution (4CN)	1 bottle	12 mL
• Substrate Buffer (SB)	1 bottle	60 mL
• Wash Buffer Concentrate (WB)	1 bottle	80 mL
• Positive Control (PC)	1 vial	0.3 mL
• Negative Control (NC)	1 vial	0.3 mL
• Wash vessels with lids		2 each

Each Kit contains an adequate quantity of reagents for a maximum of six runs. (See Section 4, Reagents, for a complete description of each reagent provided.)

B. Materials Required But Not Provided

1. Rocker (aliquot mixer) capable of maintaining between 16 to 20 rocking cycles per minute at an angle between 20 to 30 degrees.
2. Rotary shaker capable of maintaining 110 ± 5 rpm.
3. Deionized or distilled water for preparation of Working Wash Buffer and washing strips.³⁶
4. Disposable gloves.
5. Fixed or adjustable pipetting devices capable of delivering 20 µL and 1000 µL with at least ± 5% accuracy.

6. Clean glass or polypropylene graduated cylinders (10 mL, 25 mL, 50 mL, and 100 mL).
7. Serological pipettes (5 mL or 10 mL).
8. Metal or plastic forceps for handling strips.
9. Aspiration device with a vacuum source and a trap for retaining the aspirate. The aspirate from the assay is potentially infectious and should be decontaminated prior to disposal. Liquid sodium hypochlorite (i.e., household bleach) diluted 1:10 in the aspirate, or other equivalent disinfectant, can be used to disinfect the aspirate prior to disposal.
10. Assay tube rack and wash vessels (RIBA® Starter Kit, Ortho-Clinical Diagnostics, Inc., Product code 930590). Each wash vessel holds a maximum of 20 strips.

C. Preliminary Statements

1. The maximum run size is 30 strips (including a Positive and Negative Control strip); the minimum run size is 3 strips (including a Positive and Negative Control strip). Each kit contains an adequate quantity of reagents for a maximum of six runs. Each run must proceed to completion, without interruption, as specified in the Assay Procedure.
2. Assay the Positive and Negative Controls with each series of patient/donor specimens. Treat the Positive and Negative Controls exactly as patient/donor specimens throughout the Assay Procedure.
3. Verify that adequate amounts of the Working Wash Buffer and Working Substrate have been prepared before using these reagents in the assay procedure. If during the course of the Assay Procedure it is determined that not enough Working Wash Buffer or Working Substrate has been prepared to complete the Assay Procedure, the assay run is invalid and must be repeated using *one* preparation of the Working Solution(s).
4. To prevent fading, keep developed strips out of strong light (e.g., direct sunlight) and away from heat (i.e., greater than 30°C).
5. Following completion of the assay procedure, rinse the wash vessels with approximately three volumes of distilled or deionized water. Discard the wash vessels after six uses.

D. Assay Procedure

1. Approximately 30 minutes before beginning the assay, remove the kit from refrigeration (2° to 8°C) and allow the kit components to come to room temperature (15° to 30°C). Mix reagents thoroughly by gently inverting container several times before use. Avoid foaming.
2. If not already prepared, prepare Working Wash Buffer (see Table 1, Section 6, Preparation of Reagents).
3. Remove the required number of strips from the sealed foil pouches and place in the assay tube rack in their respective tubes. One tube per specimen and one tube each for the Positive and Negative kit controls are required.

Note: *Kit-supplied Positive and Negative controls must be included each time specimens are assayed. Strips must be used within two weeks after opening the pouch. Unused strips should be left in their original tubes, at 2° to 8°C, in a folded and resealed foil pouch with desiccant. Use tape to reseal pouches, and record the date the pouches were opened and the expiration date of the strips on the pouch label.*

4. Prepare a record to identify the numbers on the strips with the corresponding specimen identification numbers.
5. Remove caps from tubes and add 1 mL of Specimen Diluent to each tube (make sure that the entire strip is covered with liquid).
6. Add 20 µL of the appropriate specimen or control to the corresponding labeled tube. Cap the tubes and invert to mix.

Note: Use a new pipette tip for each specimen or control aliquoted.

7. Place the rack with the tubes on a rocker (see Figure 2) and fasten with rubber bands or tape; rock (at 16-20 cycles/minute) for 4 to 4½ hours at room temperature (15° to 30°C). Record the time started and expected ending time of the specimen incubation step.

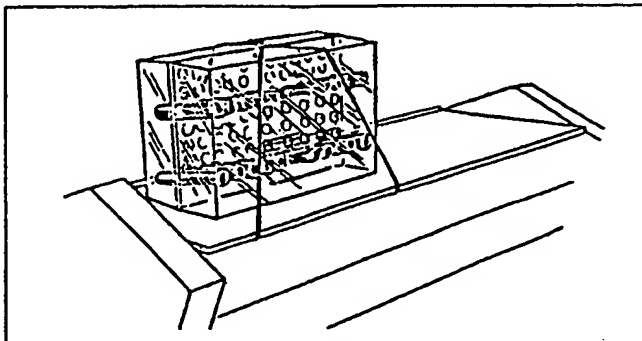


Figure 2

Note: The motion of the diluted specimen or controls over the strips, generated by a rocker, is important in achieving optimum performance of the assay. Periodically check to ensure that a rocking motion is maintained throughout the incubation. Improper functioning of the rocker, which may affect antibody binding, will invalidate the test results and require that the assay be repeated.

8. Uncap the tubes and completely aspirate liquid into an appropriate waste container (see Item 6, Section 5, Precautions). Add 1 mL of Specimen Diluent to each tube.
9. Cap the tubes and place the rack with the tubes on rocker and rock for 30 to 35 minutes at room temperature (15° to 30°C), then aspirate the liquid again.
10. Add 1 mL of Working Wash Buffer (see Table 1) to each tube, then pour liquid and strips into wash vessels containing 10 mL of Working Wash Buffer (maximum 20 strips per wash vessel). If running fewer than 20 strips, add additional Working Wash Buffer to bring the total volume to 30 mL. Swirl the strips in the wash vessel.
11. Add an additional 30 mL of Working Wash Buffer (60 mL total volume), then decant the wash, making sure the strips are retained in the wash vessel. To retain strips, gently roll the wash vessel while decanting.
12. Add 30 mL of Working Wash Buffer, swirl, add an additional 30 mL of Working Wash Buffer, then decant the wash while retaining the strips as described in Step 11.
13. Add 1 mL of Conjugate per strip to each wash vessel (minimum 10 mL per wash vessel).
14. Rotate the wash vessels on a rotary shaker at 110 ± 5 rpm for 9 to 11 minutes at room temperature (15° to 30°C).

Note: Check occasionally and shake the wash vessels to prevent strips from clumping or stacking. The circular motion is critical to the success of the assay.
15. Prepare Working Substrate (see Table 2, Section 6, Preparation of Reagents).
16. Upon completion of Conjugate incubation, decant the Conjugate. Wash the strips by adding 30 mL of Working Wash Buffer, swirl, add an additional 30 mL of Working Wash Buffer. Decant the wash and repeat this step two more times.
17. Add 1 mL of Working Substrate per strip to each wash vessel (minimum 10 mL per wash vessel).
18. Rotate the wash vessels on a rotary shaker at 110 ± 5 rpm for 15 to 20 minutes at room temperature (15° to 30°C).

19. Decant the Working Substrate, then wash the strips by adding 60 mL of DISTILLED OR DEIONIZED water and swirling. Decant the wash and repeat this step one more time. To retain strips, gently roll the wash vessel while decanting.
20. Using forceps, transfer strips to absorbent paper and blot excess water. Let strips air-dry in the dark for at least 30 minutes at room temperature. Interpret strips within 3 hours (see Section 12, Interpretation of Results).

Note: To prevent fading, keep developed strips out of strong light (e.g., direct sunlight) and away from heat (greater than 30°C).

11. Quality Control Procedures ⁴¹⁻⁴³

The assay controls supplied with the test kit must be included with each run, regardless of the number of specimens tested or strips used.

Note: A kit positive and negative control needs to be included with each assay run but not with each vessel if more than one vessel is included in the run.

The identity and location of the antigens coated on the strips are shown in Figure 3. Two levels of human IgG (Level I, low control; and Level II, high control) are included on each strip as internal controls. The reactivity of the individual antigen bands is determined by comparing the intensity of each band to the Level I and Level II human IgG internal strip controls as described in Section 12, Interpretation of Results.

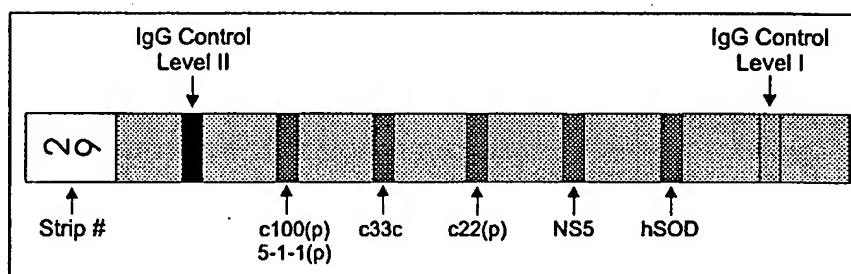


Figure 3

The following results are expected from the Positive and Negative Controls supplied with the test kit:

- a. The internal IgG control Level I and control Level II bands must be clearly distinguishable by eye on the Positive and Negative Control strips, and the IgG control Level I must be clearly lighter than the IgG control Level II.
- b. The Positive Control strip must show a response of 2+ or greater (see Interpretation of Results) for all HCV antigen and peptide bands. Response to the hSOD band must be visibly lower than the Level I human IgG control (i.e., - or +/-).
- c. The Negative Control strip must show a response to each of the HCV and hSOD antigen bands which is visibly lower than the Level I human IgG control (i.e., - or +/-).

If the assay kit controls do not meet the criteria above, then the run is invalidated and must be repeated.

Additionally, the IgG control Level I and control Level II bands must be clearly distinguishable by eye on all patient/donor specimen strips, and the IgG control Level I must be clearly lighter than the IgG control Level II. If these criteria are not met for an individual patient/donor specimen, the assay must be repeated for that specimen.

Note: *If incomplete banding, or any such artifact, on a patient/donor specimen strip hinders interpretation but the kit Positive and Negative Control strips are interpretable, the patient/donor specimen strip is invalid and the assay must be repeated for that specimen.*

12. Interpretation of Results

Anti-HCV reactivity in a specimen is determined by comparing the intensity of each antigen band to the intensity of the human IgG (Level I and Level II) internal control bands on each strip. The identity of the antibodies is defined by the specified location of the HCV band as shown in Figure 3, Section 11, Quality Control Procedures.

The intensity of the antigen/peptide bands is scored in relation to the intensities of the internal IgG controls as follows:

Intensity of Band	Score
• Absent	–
• Less than intensity of the Level I IgG control band	+/-
• Equal to intensity of the Level I IgG control band	1+
• Greater than intensity of the Level I IgG control band and less than intensity of the Level II IgG control band	2+
• Equal to intensity of the Level II IgG control band	3+
• Greater than intensity of the Level II IgG control band	4+

A NEGATIVE, INDETERMINATE, or POSITIVE interpretation is based on the reaction pattern present on the strip. For valid runs the following criteria should be used for interpretation:

Antigen Band Pattern	Interpretation
<ul style="list-style-type: none"> • No bands present having 1+ or greater reactivity, or • hSOD band alone having 1+ or greater reactivity. 	NEGATIVE
<ul style="list-style-type: none"> • Any <u>single</u> HCV band having 1+ or greater reactivity, or • hSOD band having 1+ or greater reactivity <u>in conjunction with</u> one or more HCV bands having 1+ or greater reactivity. 	INDETERMINATE
<ul style="list-style-type: none"> • At least <u>two</u> HCV bands having 1+ or greater reactivity. 	POSITIVE

A band intensity less than the IgG Control Level I (i.e., + / –) is below the cutoff for reactivity in the assay.

On rare occasions, a strip may have a dark background. If the Level I IgG and Level II IgG internal control bands are distinguishable from the background (i.e., darker than the background, with the Level II IgG control darker than the Level I IgG control), the strip is interpretable and the intensity of the antigen bands should be compared to the internal controls as described above. In anti-HCV negative specimens or specimens lacking antibodies to one or more antigens present on the strip, the antigen bands may appear lighter than the background of the strip. Such antigen bands should be interpreted as nonreactive.

13. Limitations of the Procedure

The assay must be performed in strict accordance with these instructions to obtain optimum performance.

Although the hepatitis C virus (HCV) is now known to be the primary causative agent for blood-borne non-A, non-B hepatitis (NANBH),^{11, 12} there may be causes of NANBH due to pathogens other than HCV.

The CHIRON® RIBA® HCV 3.0 SIA is limited to the detection of anti-HCV in human serum or plasma. The presence of anti-HCV is indicative of past or present infection by the hepatitis C virus, but does not always constitute a definitive diagnosis of non-A, non-B hepatitis (hepatitis C).

Specimens, such as cadaveric fluids (including serum and plasma), pleural fluid, saliva, urine, and nonhuman specimens, have not been evaluated in this assay and should not be used.

Since reactivity of 1+ or greater to any of the virus-encoded antigens on the strip is possible evidence of past or present infection with HCV, all individuals who are INDETERMINATE should be followed for at least 6 to 12 months to ascertain whether increased reactivity has occurred. It is recommended that individuals who are INDETERMINATE be retested after six months using the original specimen in addition to a freshly drawn specimen.

A specimen which is REACTIVE by a licensed anti-HCV screening procedure that is found to be NEGATIVE by CHIRON® RIBA® HCV 3.0 SIA does not exclude the possibility of infection with HCV.

Levels of anti-HCV may be undetectable in early infection.

14. Expected Results

The performance of the CHIRON® RIBA® HCV 3.0 SIA was evaluated in clinical studies of low risk, high risk, and NANBH populations. Specificity was evaluated in three populations: (1) serum specimens from normal volunteer blood donors, collected prospectively and tested at three blood centers; (2) archival specimens from deferred blood donors who were repeatedly reactive by a licensed multi-antigen screening assay; (3) specimens from individuals with liver diseases other than non-A non-B Hepatitis (NANBH). Sensitivity was evaluated using serially collected specimens from NANBH patients (seroconversion panels); specimens from patients with clinically documented NANBH; and specimens from persons at high risk for acquiring NANBH, such as hemophiliacs, long-term hemodialysis patients, and intravenous drug users (IVDU). All specimens were tested in parallel by the CHIRON® RIBA® HCV 3.0 SIA and CHIRON® RIBA® HCV 2.0 SIA.

A. Reactivity in Volunteer Random Blood Donors (Low Risk)

A total of 3004 sequential, previously unscreened, random blood donations were collected prospectively and tested at three blood centers participating as clinical sites. Table 3 summarizes the results. Of these 3004 donors, 5 were positive by both assays and 2955 were negative by both assays. Twenty-nine specimens were indeterminate by CHIRON® RIBA® HCV 3.0 SIA only and 9 were indeterminate by CHIRON® RIBA® HCV 2.0 SIA only. The specificity of CHIRON® RIBA® HCV 2.0 SIA was 99.5% (2984/2999) and the specificity of CHIRON® RIBA® HCV 3.0 SIA was 98.8% (2964/2999).¹

¹Specificity was calculated as follows: $\frac{TN}{TN + FP}$

where TN = true negatives, that is, the number of specimens negative by CHIRON® RIBA® SIA; and FP = false positives, that is, the number of specimens indeterminate by CHIRON® RIBA® SIA.

Table 3. CHIRON® RIBA® HCV 2.0 SIA and CHIRON® RIBA® HCV 3.0 SIA Testing Results in Volunteer Blood Donors from Three Blood Centers (Low Risk)

CHIRON® RIBA® HCV 3.0 SIA	CHIRON® RIBA® HCV 2.0 SIA			
	Positive	Indeterminate	Negative	TOTAL
Positive	5	0	0	5 (0.2%)
Indeterminate	0	6	29	35 (1.1%)
Negative	0	9	2955	2964 (98.7%)
TOTAL	5 (0.2%)	15 (0.5%)	2984 (99.3%)	3004 (100%)

B. Reactivity in Volunteer Blood Donor Specimens Repeatedly Reactive by a Licensed Multi-Antigen Anti-HCV Screening Procedure

In two separate studies, the CHIRON® RIBA® HCV 3.0 SIA and CHIRON® RIBA® HCV 2.0 SIA were evaluated with a total of 851 specimens which were repeatedly reactive by licensed Version 2.0 or Version 3.0 multi-antigen anti-HCV screening procedures.

In the first study, a total of 732 specimens that were repeatedly reactive by licensed Version 2.0 multi-antigen screening procedures were tested with the CHIRON® RIBA® HCV 3.0 SIA and CHIRON® RIBA® HCV 2.0 SIA. The results of these studies are shown in Table 4. Of these 732 specimens, a larger number were negative by CHIRON® RIBA® HCV 3.0 SIA than by CHIRON® RIBA® HCV 2.0 SIA, and a smaller number were found to be indeterminate by CHIRON® RIBA® HCV 3.0 SIA than by CHIRON® RIBA® HCV 2.0 SIA.

Table 4. Comparison of CHIRON® RIBA® HCV 3.0 SIA and CHIRON® RIBA® HCV 2.0 SIA in Specimens Repeatedly Reactive by a Licensed Version 2.0 Multi-antigen Anti-HCV Screening Procedure

CHIRON® RIBA® HCV 3.0 SIA	CHIRON® RIBA® HCV 2.0 SIA			
	Positive	Indeterminate	Negative	TOTAL
Positive	432	26	2	460 (62.8%)
Indeterminate	2	34	18	54 (7.4%)
Negative	2	165	51	218 (29.8%)
TOTAL	436 (59.6%)	225 (30.7%)	71 (9.7%)	732 (100%)

In the second study, a total of 119 specimens which were repeatedly reactive by a licensed Version 3.0 multi-antigen screening procedure were tested with the CHIRON® RIBA® HCV 3.0 SIA and CHIRON® RIBA® HCV 2.0 SIA. These 119 specimens represent an unscreened donor population of approximately 30,000 individuals. The results of this study are given in Table 5. Of these specimens, a greater number were found to be either positive or indeterminate by CHIRON® RIBA® HCV 3.0 SIA than by CHIRON® RIBA® HCV 2.0 SIA.

Table 5. Comparison of CHIRON® RIBA® HCV 3.0 SIA and CHIRON® RIBA® HCV 2.0 SIA in Specimens Repeatedly Reactive by a Licensed Version 3.0 Multi-antigen Anti-HCV Screening Procedure

CHIRON® RIBA® HCV 3.0 SIA	CHIRON® RIBA® HCV 2.0 SIA			
	Positive	Indeterminate	Negative	TOTAL
Positive	78	3	0	81 (68.1%)
Indeterminate	0	11	12	23 (19.3%)
Negative	0	3	12	15 (12.6%)
TOTAL	78 (65.5%)	17 (14.3%)	24 (20.2%)	119 (100%)

C. Specificity in Specimens from Individuals with Other Liver Diseases or Elevated Levels of Immunoglobulins

Two study sites tested a total of 364 specimens from persons with initial diagnoses of liver diseases other than NANBH. These included HAV, acute and chronic HBV, CMV, autoimmune hepatitis, nonviral liver disease, and specimens with elevated IgA, IgM, or IgG. Results are presented in Table 6. There were only three specimens with discrepant results between CHIRON® RIBA® HCV 2.0 SIA and CHIRON® RIBA® HCV 3.0 SIA. Two of these specimens were negative by a licensed multi-antigen anti-HCV screening procedure; the other was repeatedly reactive.

Table 6. CHIRON® RIBA® HCV 2.0 SIA and CHIRON® RIBA® HCV 3.0 SIA Results in Specimens from Subjects with Other Liver Diseases

CHIRON® RIBA® HCV 3.0 SIA	CHIRON® RIBA® HCV 2.0 SIA			
	Positive	Indeterminate	Negative	TOTAL
Positive	9	0	0	9 (2.5%)
Indeterminate	0	3	3	6 (1.7%)
Negative	0	0	349	349 (95.8%)
TOTAL	9 (2.5%)	3 (0.8%)	352 (96.7%)	364 (100%)

D. Sensitivity in Seroconversion Panels

A total of 86 seroconversion panels from individuals with documented seroconversion to antibodies to HCV and clinical documentation of NANBH were tested. Subjects were categorized as acute if two sequential serum specimens had SGPT/ALT levels greater than 44 IU and 90 IU, respectively, and the SGPT/ALT level returned to normal within six months. Subjects whose SGPT/ALT level remained greater than two times the upper limit of normal for longer than six months were categorized as having chronic NANBH. All subjects diagnosed as having NANBH were serologically negative for hepatitis A and hepatitis B. Table 7 summarizes the CHIRON® RIBA® HCV 2.0 SIA and CHIRON® RIBA® HCV 3.0 SIA results from these panels.

Table 7. Summary of Seroconversion Panel Testing Results

Category of NANBH	N	Equal Sensitivity/ Equal Reactivity*	Equal Sensitivity/ CHIRON® RIBA® HCV 3.0 SIA Greater Reactivity	CHIRON® RIBA® HCV 3.0 SIA Greater Sensitivity
Acute	32	13	9	10
Chronic	41	14	10	17
Indeterminate	9	3	2	4
Not Specified	4	4	0	0
TOTAL	86 (100%)	34 (40%)	21 (24%)	31 (36%)

* The term "sensitivity" refers to any reactivity (indeterminate or positive) vs no reactivity; "reactivity" refers to the magnitude of reactivity (indeterminate vs positive).

In 52 of the 86 panels (60%), CHIRON® RIBA® HCV 3.0 SIA showed greater reactivity and/or sensitivity than CHIRON® RIBA® HCV 2.0 SIA. In 21 panels (24%), both assays detected anti-HCV on the same blood draw, but CHIRON® RIBA® HCV 3.0 SIA became positive earlier than CHIRON® RIBA® HCV 2.0 SIA (i.e., greater reactivity). In 31 panels (36%), CHIRON® RIBA® HCV 3.0 SIA detected anti-HCV earlier than CHIRON® RIBA® HCV 2.0 SIA, with a mean difference of 50 days (range 9 to 282 days) between detection by CHIRON® RIBA® HCV 2.0 SIA and CHIRON® RIBA® HCV 3.0 SIA. In no case was CHIRON® RIBA® HCV 3.0 SIA less sensitive/reactive than CHIRON® RIBA® HCV 2.0 SIA.

E. Sensitivity in Patients with Acute and Chronic NANBH

A total of 239 specimens from patients with documented acute NANBH and 96 specimens from patients with documented chronic NANBH were evaluated.

The summary results from testing the acute NANBH specimens are shown in Table 8. These subjects had the following clinical findings: a serum SGPT/ALT level of greater than 500 IU/L (site 1) or 1000 U/mL (site 2); no reported use of hepatotoxins; and negative serology for anti-HAV IgM, anti-HBc IgM, and HBsAg. A greater number of the acute NANBH specimens were reactive (i.e., positive or indeterminate) by CHIRON® RIBA® HCV 3.0 SIA than by CHIRON® RIBA® HCV 2.0 SIA (187 and 174, respectively), and CHIRON® RIBA® HCV 3.0 SIA provided a more definitive diagnosis (positive or negative vs indeterminate) than CHIRON® RIBA® HCV 2.0 SIA. Of the 173 specimens that were reactive by both assays, CHIRON® RIBA® HCV 3.0 SIA was positive in 156 (89%) compared to 132 (76%) for CHIRON® RIBA® HCV 2.0 SIA.

Table 8. CHIRON® RIBA® HCV 2.0 SIA and CHIRON® RIBA® HCV 3.0 SIA Results on Specimens from Patients with Acute NANBH

CHIRON® RIBA® HCV 3.0 SIA	CHIRON® RIBA® HCV 2.0 SIA			
	Positive	Indeterminate	Negative	TOTAL
Positive	131	23	2	156 (65.2%)
Indeterminate	1	18	12	31 (13.0 %)
Negative	0	1	51	52 (21.8%)
TOTAL	132 (55.2%)	42 (17.6 %)	65 (27.2 %)	239 (100%)

Table 9 presents the summary testing results on 96 specimens from patients with documented chronic NANBH. These subjects demonstrated persistently elevated SGPT/ALT levels (greater than two times the upper level of normal) for more than six months, no history of hepatotoxin abuse, and were negative for HBsAg. The results showed no differences in sensitivity or reactivity between CHIRON* RIBA* HCV 2.0 SIA and CHIRON* RIBA* HCV 3.0 SIA in patients with chronic NANBH.

Table 9. CHIRON* RIBA* HCV 2.0 SIA and CHIRON* RIBA* HCV 3.0 SIA Results in Specimens from Chronic NANBH Patients

CHIRON* RIBA* HCV 3.0 SIA	CHIRON* RIBA* HCV 2.0 SIA			
	Positive	Indeterminate	Negative	TOTAL
Positive	83	0	0	83 (86.5%)
Indeterminate	0	1	0	1 (1.0%)
Negative	0	0	12	12 (12.5%)
TOTAL	83 (86.5%)	1 (1.0%)	12 (12.5%)	96 (100%)

F. Sensitivity in High Risk Populations

The performance of the CHIRON* RIBA* HCV 2.0 SIA and CHIRON* RIBA* HCV 3.0 SIA was evaluated with a total of 614 specimens collected from members of high risk groups, comprising hemophiliac patients, hemodialysis patients, and intravenous drug abusers. The results are shown in Table 10. A greater number of these specimens were reactive by CHIRON* RIBA* HCV 3.0 SIA than by CHIRON* RIBA* HCV 2.0 SIA (417 vs 408, respectively). As in the acute NANBH population, a higher percent of the reactive specimens were positive by CHIRON* RIBA* HCV 3.0 SIA (96.4%, 402/417) than by CHIRON* RIBA* HCV 2.0 SIA (94.1%, 384/408).

Table 10. CHIRON* RIBA* HCV 2.0 SIA and CHIRON* RIBA* HCV 3.0 SIA Results in High Risk Populations

CHIRON* RIBA* HCV 3.0 SIA	CHIRON* RIBA* HCV 2.0 SIA			
	Positive	Indeterminate	Negative	TOTAL
Positive	383	14	5	402 (65.5%)
Indeterminate	1	9	5	15 (2.4%)
Negative	0	1	196	197 (32.1%)
TOTAL	384 (62.5%)	24 (3.9%)	206 (33.6%)	614 (100%)

15. Specific Performance Characteristics

A. Potentially Interfering Substances and Conditions

The effect of elevated levels of triglycerides, bilirubin, and hemoglobin were evaluated in the CHIRON® RIBA® HCV 3.0 SIA using anti-HCV positive and anti-HCV negative specimens. The effect of microbial contamination was also evaluated, using specimens spiked to a final concentration of 10^3 CFU/mL with *Candida albicans*, *Pseudomonas aeruginosa*, or *Staphylococcus epidermidis*. The microbially contaminated specimens were tested on the day of spiking, at Day 3, and again at Day 8. The effect of multiple freeze-thaws and heat inactivation were also evaluated. The results are presented in Table 11. Assay results were comparable under all conditions tested.

Table 11. Effect of Potentially Interfering Substances and Conditions on CHIRON® RIBA® HCV 3.0 SIA

Substance or Condition	Level of Interferent or Description of Condition	Effect
Triglycerides	up to 1600 mg/dL	No Effect
Bilirubin	up to 60 mg/dL	No Effect
Hemoglobin	up to 80 mg/dL	No Effect
Microbial contamination	10^3 for up to 8 days	No Effect
Freeze-thaws	5 cycles	No Effect
Heat inactivation	56°C for 1 hour	No Effect
Anti-yeast antibodies	4 specimens	No Effect
Anti- <i>E. coli</i> antibodies	22 specimens	No Effect

B. Specimen Collection Devices

The performance of the CHIRON® RIBA® HCV 3.0 SIA was evaluated in anti-HCV positive and anti-HCV negative specimens collected in serum Vacutainer® tubes, serum separator tubes, and in the following anticoagulants: K₂ EDTA (15% solution), ACD (Solution B), sodium heparin, CPDA-1, and 4% sodium citrate. An additional study compared the pilot (serum) tube specimen to the specimen from the blood bag segment. Assay results were comparable with all specimen collection devices and anticoagulants tested.

C. Analytical Sensitivity

A dilutional analysis of 10 anti-HCV positive specimens was performed comparing results from the CHIRON® RIBA® HCV 3.0 SIA and the CHIRON® RIBA® HCV 2.0 SIA. Individual antigen band results were evaluated to determine the lowest detectable dilution for each band for each test. For each antigen band of each specimen, the CHIRON® RIBA® HCV 3.0 SIA had the same or greater dilutional sensitivity as the CHIRON® RIBA® HCV 2.0 SIA. The percent of antigen band determinations with greater dilutional sensitivity using the CHIRON® RIBA® HCV 3.0 SIA was 40% for the c22 band, 50% for the c100 band, and 80% for the c33c band. In no case was the dilutional sensitivity of CHIRON® RIBA® HCV 3.0 SIA less than that of CHIRON® RIBA® HCV 2.0 SIA.

D. Reproducibility

The precision of the CHIRON* RIBA* HCV 3.0 SIA was established in two studies, one that assessed assay reproducibility across assay runs, operators, sites, and lots; the second that assessed the reproducibility of antigen band and strip interpretations under different lighting conditions. Both studies utilized the same six-member panel, which included three positive specimens, two indeterminate specimens, and one negative specimen.

In the first study, three operators at each of three sites performed the testing. Each operator tested the panel in singlicate in three different runs using each of three kit lots. Therefore, for each of the three kit lots, there were a total of 27 CHIRON* RIBA* HCV 3.0 SIA strips tested with each panel member. In the study, no strips were incorrectly interpreted. In addition, 492 of 500 (98.4%) antigen band ratings were within one level of intensity of the consensus result. Five of the 8 discordant ratings were +/- when the consensus reading was 2+. The other three cases involved band ratings of 4+ when the consensus reading was 2+. The majority of the variability in band interpretation was attributable to within-run and between-run components. These data demonstrate that the scoring of the intensity of CHIRON* RIBA* HCV 3.0 SIA antigen bands is reproducible across multiple sites, operators, and lots.

At one site, the reproducibility panel was tested to assess the effect of different lighting conditions (fluorescence, incandescence, or natural lighting) on interpretation of the CHIRON* RIBA* HCV 3.0 SIA strips. Each panel member was read a total of 27 times under each of the three lighting conditions, for a total of 81 readings. Regardless of lighting condition, all panel members were interpreted correctly by all readers with all kit lots. The interpretation of individual antigen bands for each panel member was also consistent from one lighting condition to another. The data demonstrated that scoring of intensity of the CHIRON* RIBA* HCV 3.0 SIA was not affected by lighting conditions.

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